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Diffusion Tensor Imaging of Healthy and Infarcted Porcine Hearts: Study on the Impact of Formalin Fixation

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ABSTRACT

Background: Because of the complexities with in-vivo cardiac diffusion tensor imaging (DTI), ex-vivo formalin-fixed specimens are used to investigate cardiac remodeling in diseases, and reported results have shown conflicting trends. This study investigates the impact of formalin fixation on diffusion properties and optimizes tracking parameters based on controls to understand remodeling in myocardial infarction (MI).

Methods: DTI was performed on four healthy (controls) and four MI-induced, formalin-fixed (PoMI) ex-vivo porcine hearts. Controls were scanned before fixation (PrCtrl) and rescanned (PoCtrl) after formalin fixation. Fractional anisotropy (FA) and apparent diffusion coefficient (ADC) were estimated in all hearts. Tracking parameters (FA, tract termination angle [TTA], and fiber length) were optimized in controls and then used to investigate structural remodeling in PoMI hearts.

Results: Fixation increased ADC and decreased FA. PoMI showed increased ADC but decreased FA in the infarcted region compared to the remote region. TTA showed a sharp increase in slope from 5° to 10°, which flattened after 25° in all groups. Mean fiber length for different tracking length ranges showed that PoCtrl had shorter fibers compared with PrCtrl. Fibers around the infarction were shorter in length and disarrayed compared to the PoCtrl group.

Conclusion: Formalin fixation affects diffusion properties; hence, DTI parametric trends observed in pathology may be influenced by the fixation process, which can cause contradictory findings.

RÉSUMÉ

Contexte : En raison de la complexité de l'imagerie in-vivo du tenseur de diffusion (DTI) cardiaque, des spécimens ex-vivo fixés dans Le formol sont utilisés pour l'étude du remodelage cardiaque dans la maladie, et les résultats rapportés affichent des tendances conflictuelles. La présente étude examine l'effet de la fixation au formol sur les propriétés de diffusion et optimise les paramètres de suivi basés sur les témoins, afin de comprendre le remodelage dans l'infarctus du myocarde (MI).

Méthodologie : Une DTI a été effectuée sur quatre sujets (cœurs de porcs) en bonne santé (témoins) et quatre sujets ports ex-vivo ayant subi un IM induit fixés au formol (PoMI). Les témoins ont été imagés avant la fixation (PrCtrl) et imagés de nouveau (PoCtrl) après la fixation au formol. L'anisotropie fractionnaire (FA) et le coefficient de diffusion apparent (ADC) ont été estimés pour tous les cœurs. Les paramètres de suivi (FA, angle de terminaison du tractus (TTA), longueur des fibres) ont été optimisés pour les témoins et utilisés pour étudier le remodelage structural dans les cœurs PoMI.

Résultats : La fixation augmente l'ADC et diminue la FA. Les cœurs PoMI présentent une augmentation de l'ADC mais une diminution de la FA dans les zones d'infarctus comparativement aux zones plus éloignées. Le TTA affiche une forte progression de la pente entre 5o-10°, avec un aplanissement après 25° dans tous les groupes. La longueur de fibre moyenne pour les différentes plages de longueur de suivi montre que les images PoCtrl présentent des fibres plus courtes en comparaison des images PrCtrl. Les fibres entourant la zone infarcie étaient plus courtes et en désordre comparativement au groupe PoCtrl.

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Conclusion : La fixation au formol affecte les propriétés de diffusion; par conséquent, les tendances des paramètres de DTI observées en pathologie pourraient être influencées par le processus de fixation, ce qui peut entraîner des conclusions contradictoires.

Keywords: Myocardial infarction; diffusion tensor imaging; formalin fixation; remodeling

Introduction

Cardiovascular diseases continue to be the leading cause of mortality and morbidity worldwide. Among different types of cardiovascular diseases, myocardial infarction (MI), characterized by cellular necrosis of a segment of the heart muscle, is a major and well-recognized contributor. In the United States alone, approximately 6.4 million patients are hospitalized annually for initial occurrence of MI and 2.8 million for MI recurrence [1].

Histologic evidence has shown that MI causes remodeling (change in shape, size, and function) of the left ventricular (LV) myocardium [2–4]. This remodeling adversely affects cardiac function and, if untreated, eventually leads to heart failure [5, 6]. Therefore, there is a need to understand the effect of MI on LV remodeling associated with the changes in the fiber structure and orientation, which could potentially facilitate novel treatment development for MI.

Conventional ex-vivo histologic approaches to delineate MI have been replaced by advanced in-vivo imaging [7–11]. Cardiac magnetic resonance imaging (MRI) with delayed enhancement imaging [12–15] is highly accurate in detecting MI, and myocardial tagging [16–18] can quantitatively determine the decrease in contractile function in an infarcted region. With the advent of cardiac diffusion tensor imaging (DTI) [19–27], an MRI-based technique, researchers have been able to noninvasively investigate the three-dimensional myocardial fiber architecture in an MI model.

DTI is based on the Brownian motion exhibited by water molecules in biological tissues, which is known to be impeded or facilitated based on the tissue structure and its orientation [28]. This inherent property of diffusion demonstrated by water molecules causes an exponential decay of the received MR signal that can be characterized using a 3×3 diffusion tensor D [29].

Diagonalization of D provides the eigenvalues (λ_1, λ_2, and λ_3) and eigenvectors (ε_1, ε_2, and ε_3) of the tensor. These eigenvalues and vectors completely define the anisotropic diffusion occurring at each imaging voxel. The principal eigenvector (ε_1) corresponds to the direction of maximum diffusivity which is aligned in the direction of the fiber; whereas the other two vectors (ε_2, ε_3) relate to the direction of radial diffusivity. D can be used to analyze the myocardial fiber architecture [30] and provide quantitative structural information using parameters such as fractional anisotropy (FA), trace values, apparent diffusion coefficient (ADC) [29], and helical angles [31]. The myocardial fiber architecture thus established with DTI has been validated against previously established histologic models [31, 32].

Recently, cardiac DTI has been implemented in-vivo in humans [19] and in patients [27, 33, 34] to study normal and pathologic fiber architecture. However, high-resolution cardiac DTI is still under development because of difficulties associated with in-vivo imaging (sensitivity of DTI imaging parameters to cardiac and respiratory motion) and therefore, to date, most studies performed to establish myocardial architecture using cardiac DTI have been implemented on ex-vivo animal specimens. These ex-vivo specimens are generally fixed in formalin after extraction to preserve and maintain the tissue. Therefore, there is a need to understand the impact of formalin fixation on the diffusion properties of the myocardium so as not to misinterpret the findings as being related to the pathology of concern.

In addition, ex-vivo cardiac DTI has been explored by various groups to study remodeling after MI. FA, trace values, ADC, and helical angles have been examined across the myocardial wall in regions remote from, adjacent to, and within the infarcted area in different animal models [35–42]. However, different study groups have reported contradictory results with respect to the effect on DTI parameters as a result of MI [36, 38, 42]. The underlying truth behind how the actual pathology alters the DTI parameters and how much of it is a result of the fixation process remains arguable and needs to be verified before any substantial claim is made. We hypothesize that the conflicting results in terms of how diffusion parameters are affected because of MI can be attributed to the process of fixation and its effect on the diffusion properties of water molecules. The aim of this study is to investigate the effect of myocardial tissue fixation on DTI parameters (ADC, FA, tract termination angle [TTA] and fiber length) by comparing healthy ex-vivo prefixed (PrCtrl) hearts to postfixed (PoCtrl) hearts in a porcine model. Furthermore, the study also investigates remodeling of the fiber architecture with respect to fiber length in a fixed, infarcted (PoMI) porcine model by using optimized DTI tracking parameters obtained from comparison of the control groups before and after fixation.

Methods

Eight juvenile Yorkshire pigs (90–110 lbs) were used in this study in compliance with the Ohio State University's Institutional Animal Care and Use Committee. Four pigs were used as controls (PrCtrl and PoCtrl) to study the effect of formalin fixation on the diffusion parameters and fiber architecture. MI was induced in the other four pigs (PoMI) to study the remodeling of fiber architecture.